Minutes of the Steering Committee: 03-06-03

Attending:

Adam Arkin
Kristin Balder-Froid
Terry Hazen
Jay Keasling
Vince Martin
Anup Singh
David Stahl
Judy Wall
Joe Zhou

Progress Report Notes

1. Adam
    i. Urged people to post to BioFiles and the Discussion Board
    ii. Discussed the oligo chips for Desulfovibrio
        1. Told him to download sequence from BioFiles
        2. We talked about making the oligo chips.
2. Judy want to bring in Garrit Voordal from Calgary as the best Desulfogeneticist around.
    a. Talked about getting his mutants and collaboration.
    b. Talked about submitting a GTL II proposal
    c. Joe talked about looking at his mutants
3. Judy now has it's bioreactor and it's not quite set up yet. It will be a couple of more weeks before the operation. Her post-doc Bill Yen will be ready to work after surgery at about that point. She is cloning and preparing the heat-shock proteins for Anup. Also doing dnaK but there is a problem with the restriction sites in the tag vector and those that occur in the protein. They've tried alternative sites but it's not working. They are going to try hspC as well.
4. Anup will take those proteins and try and pull out the proteins associated with it. Starting with these E. coli heat-shock protein homologs with strep tags will bring the complexes down with IP, purify with SDS page, and put into mass spec. In the meantime, without mutants, without the genetic tags they are trying to use antibodies to E. coli on the D.v. protein. They've done their first heat shock experiments and their growth curve (now their doubling twice as fast as what Judy reported. Eek.). The experiment was growing the cells to mid-log phase, did the heat shock (50C) experiment in quadruplicate and took a time course. Looking for changes in genome wide proteome. Anup is also doing UV (4W @355nm) shock to E. coli and D.V. and see if there is any difference in mass-spec. There experiments have been done and just waiting for time on the mass-spec—about two weeks.

a. Judy wants to know the survival rate at the temperature to control for multiple effects.

5. Jay and Vince. Just got in the nitrogen glove box for D.v. culturing. The metabolomic analysis of E. coli is coming along with the Co-A analysis. They now have an array of Co-A's and ATP, ADP and AMP and are moving towards ore metabolites. Also working on methods for stopping metabolism quickly. Developed new centrifuge tubes and a silicon oil method—the cell spin through the silicon oil and het the TCA and lyse. The supernatant from the growth medium stays at the top. Cells concentrate through the silcon oil and metabolism is stopped in the cold TCA. This is better than the cold methanol stops. By looking at the Co-A's and the ATPS, this method is much better. There is a ml of reaction volume but they are trying to reduce this. Vince has also collected all the summaries for the lab.
6. David Stahl
    a. Sergei Stolyer and another visited LBL and got trained.
    b. Received their first core samples from the FRC sites.
    c. Previously obtained a 6 mo old core from FRC
        i. Set up a most-probable-number (MPM) series on the sample (nutrient broth, acetate/lactate, etc.). It's been running for two weeks. And will extend to proprionate and H2CO2
        ii. Will begin with the fresh batch shortly.
        iii. Discussed with John Leigh and Barney Woodman doing the first complex community assessment but co-culturing with a methanogen. They were very positive and will get moving right away and will begint o examine salinity conditions for the two organisms (the methanogen is marine). This should be straightforward. (The methanogen array is being built by Barney and John). Martin also though his proteomics system could work on the co-culture.
        iv. Now looking at 20% heavy metal impacted waste-water sites. Observed sulfate respiration becomes dominant as you move up the heavy-metal gradient. They have lactate/sulfate grown isolates from this site…they will do the phylogenetic analysis and decide whether to send them on to LIC.
        v. Defrosted other strains of Desulfo (PT2, desulfericans, etc.). PT2 also co-cultures with methanogens and might be interesting for the comparative studies. It's below 30% DNA homology but still pretty related.
        vi. Reactors are still on back-order so that should be happening pretty soon.
7. Joe Zhou: Moving on to choosing 70-mers Compared 50,60 and 70 bp oligos and did spotting experiments and found. For Shewanella, they've done heat shock and had problems (because of the inability to get homogeneous temperature). The salt and pH stress gave good data. The interaction traps are stalled because of problems of amplifying the Shewanella ORFS because Taq is too error prone. They have done some optimization and now they found that the they need a LOT

of enzyme ($100K for doing the whole genome). Yikes. They are ready to go if they can do a small amount. A new post-doc will also be showing up around august.

8. Terry got the FRC sample in last Thursday. Martin and Dave already have older samples. They are from all three FRC contaminated areas but none of those samples have uranium more than 25 ppm which puts them below much of the regulatory radars. They were shipped under gas so they should remain anaerobic. Shipped a kg of each type of sediment to Dave and almost shipped to Martin but Martin can't receive ANY contaminated soil so he may have to work up here.
    a. Hoi-Yin has been looking at oxygen stress in Shewanella and D.v. and saw that on the first exposure to air there was DNA damage to document and could see the cells start to repair themselves. After a week of exposure not only are the cells still alive but they modify their cell membrane which has thickened in some way and the cells maintain activity. (All using Synchrotron FTIR). Judy suggests looking at the cells under EM and Terry said Hoi-Ying will actually do it. Judy asked Terry if the cells are forming films on the gold plate that Hoi-Ying is using. Terry such films form but that they don't look like standard biofilms.
    b. Terry is trying to optimize biomass production in chemostats and attached reactors. He would like to compare the two results.